ENVIRONMENTAL FATE/PATHWAYS: ROBUST SUMMARIES

PHOTODEGRADATION

REPORT NUMBER: PD-1

STUDY TYPE: Photodegradation

TEST MATERIAL: Butyl Glycidyl Ether; CAS # 2426-08-6

STUDY NUMBER(S): Not applicable

SPONSOR: Air Products and Chemicals, Inc.

TESTING FACILITY: Air Products and Chemicals, Inc.

TITLE OF REPORT: Not applicable

AUTHOR(S): Not applicable

REPORT ISSUED or COMPLETION DATE: August 23, 2001

RECOGNIZED METHOD, i.e OECD: Modeling conducted; no guideline studies used.

GLP: Not utilized

METHOD: Estimation Programs Interface for Microsoft® Windows (EPIWIN V3.05, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, D.C.), Atmospheric Oxidation Program (v1.90) modeling component. Model executed in August 2001.

RESULTS/OBSERVATIONS: The AOP component of EPIWIN was used to calculate the rate of photodegradation for n–butyl glycidyl ether. Results suggest n-butyl glycidyl ether readily absorbs solar radiation and undergoes photochemical degradation; the half-life was calculated to be 0.539 days (or 6.466 hours). This assumes the reaction occurred over a 12-hour day with an average atmospheric concentration of 1.5E6 OH/cm^3.

DATA QUALITY: Data was generated by a scientific model based on structure-activity relationships that are well documented and acceptable for use in environmental assessments.

RELIABILITY:

1 w/o restriction	[ ]
2 w restriction	[X]
3 not reliable	[ ]
4 not assignable	[ ]

Reason for Reliability Code: Reference is a scientific model.

TRANSPORT/DISTRIBUTION (FUGACITY)

REPORT NUMBER: TD-1

STUDY TYPE: Transport/distribution between environmental compartments (fugacity)

TEST MATERIAL: Butyl Glycidyl Ether; CAS # 2426-08-6

STUDY NUMBER(S): Not applicable

SPONSOR: Air Products and Chemicals, Inc.

TESTING FACILITY: Air Products and Chemicals, Inc.

TITLE OF REPORT: Not applicable

AUTHOR(S): Not applicable

REPORT ISSUED or COMPLETION DATE: August 23, 2001

RECOGNIZED METHOD, i.e OECD: Modeling conducted; no guideline studies used.

GLP: Not utilized

METHOD: Estimation Programs Interface for Microsoft® Windows (EPIWIN V3.05, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, D.C.), LEV3EPI fugacity model used to predict partitioning among air, water, sediment and soil. Model executed in August 2001.

RESULTS/OBSERVATIONS: The following are the results from the LEV3EPI model for predicting partitioning of n-butyl glycidyl ether among air, water, soil and sediment. Results were obtained by running a single level III output using the LEV3EPI model default emission rates of 1000 kg/hr for air, water and soil. The advection times used were also the LEV3EPI model default values. The half-life values used were those calculated by BIOWIN and AOPWIN programs. The LEV3EPI model used the experimental log Kow value from the KOWWIN program to calculate the soil Koc value.

Level III Fugacity Model (Full-Output):
Chem Name: Oxirane, (butoxymethyl)-
Molecular Wt: 130.19
Henry's LC: 4.37e-006 atm-m3/mole (Henrywin program)
Vapor Press: 2.19 mm Hg (Mpbpwin program)
Log Kow: 0.63 (Kowwin program)
Soil Koc: 1.75 (calc by model)

LEV3EPI Compartmental Distribution from EPIWIN V3.05 Fugacity Calculation

Media		LEV3EPI Default Values	
	Half-Life (hr)	Emissions (kg/hr)	Concentration, Percent
Air	12.9	1000	2.11
Water	360	1000	53.5
Soil	360	1000	44.3
Sediment	1.44e+003	0	0.0924

Data From LEV3EPI Default Input Emissions

	Fugacity (atm)	Reaction (kg/hr)	Advection (kg/hr)	Reaction (percent)	Advection (percent)
Air	3.16e-010	901	168	30	5.6
Water	7.17e-010	822	427	27.4	14.2
Soil	1.93e-010	682	0	22.7	0
Sediment	5.94e-010	0.355	0.0148	0.0118	0.000492

	LEV3EPI Default
Persistence Time:	266 hr
Reaction Time:	332 hr
Advection Time:	1.34e+003 hr
Percent Reacted:	80.2
Percent Advected:	19.8

Half-Lives (hr) (based upon BIOWIN [Ultimate] and AOPWIN):
Air: 12.93
Water: 360
Soil: 360
Sediment: 1440
BIOWIN estimate: 3.192 (weeks)

Advection Times (hr) (based on LEV3EPI default values):
Air: 100
Water: 1000
Sediment: 5e+004

DATA QUALITY: EPIWIN V3.05 data are predictive estimates from LEV3EPI model developed by the EPA Office of Pollution Prevention and Toxics and Syracuse Research Corporation. Estimates from the model are reliable for estimating partitioning among environmental compartments based on the input parameters.

RELIABILITY:

1 w/o restriction [ ]
2 w restriction [X]

3 not reliable [ ]
4 not assignable [ ]

Reason for Reliability Code: Reference is a scientific model.

BIODEGRADATION

REPORT NUMBER: B-1

STUDY TYPE: Biodegradation

STUDY MATERIAL: n-Butyl glycidyl ether (1-Butoxy-2,3-epoxypropane); CAS No. 2426-08-6; BATCH: 696973

STUDY NUMBER: STL Ref No: ST 80/056; SBGR.82.069

SPONSOR: SICC/CSAS

TESTING FACILITY: Shell Biosciences Laboratory, Sittingbourne Research Center

TITLE OF REPORT: n-Butyl glycidyl ether: Assessment of Ready Biodegradability

AUTHOR(S): C.M. Stone and R.J. Watkinson

RECOGNIZED TEST METHOD: OECD Test Guidelines 301B (Modified Sturm) and 301D (Closed Bottle Test).

TEST TYPE: Closed Bottle Test, Modified Sturm Test

GLP: Yes

YEAR: 1982

CONTACT TIME: 28 days

INOCULUM: Test micro-organisms were obtained from Sturry sewage works.

REMARKS: Closed Bottle Test: The test substance was added to the test medium from a stock aqueous solution of 1.040 g/l to give a concentration of 3.0 mg/l and dispensed into BOD bottles. The bottles were incubated at 21+/-1°C and the extent of biodegradation determined by measuring oxygen concentration in the bottles at 0, 5, 15, and 28 days.

Modified Sturm Test: The test substance was added to the test medium from a stock aqueous solution of 1.040 g/l to give a concentration of 21 mg/l, dispensed into Sturm vessels and

inoculated and aerated with 60 ml/min of CO_2-free air. Extent of biodegradation at days 2, 4, 7, 9, 28 and 29 days was determined by titrating the total carbon dioxide evolved in the incubation.

RESULTS: The results of the closed bottle test showed n-butyl glycidyl ether oxidized to 25% of the theoretical oxygen demand by day 28. For the modified Sturm test duplicate incubations showed 4 and 12% degradation compared to theoretically possible carbon dioxide.

DATA QUALITY: Study was conducted in accordance with a recognized scientific procedure for determining biodegradability. The study meets national and international scientific methods and provides sufficient information to support the conclusion.

RELIABILITY:

1. w/o restriction	[x]
2. w restriction	[ ]
3. not reliable	[ ]
4. not assignable	[ ]

REFERENCES:

OCED Guidelines for Testing of Chemicals, 1981.

Final Report of the OCED Expert Group on Degradation /Accumulation, 1980.

Cox, D.F. 1978. Ann. Rev. Microbiol 23, 173-194.

ECOTOXICITY: ROBUST SUMMARIES

ACUTE TOXICITY TO FISH

REPORT NUMBER: AF-1

STUDY TYPE: Acute toxicity to fish

TEST MATERIAL: n-butyl glycidyl ether; CAS # 2426-08-6; Supplied by: Shell Chemicals UK, Stanlow; Lot/Batch No: 696973; Solubility: Reported to be sufficiently soluble for all stock solutions to be prepared in distilled water.

STUDY NUMBER(S): SBGR.82.148

SPONSOR: SICC/CSAS; Shell Oil Company

TESTING FACILITY: Shell Toxicology Laboratory (Tunstall), Sittingbourne Research Center, Kent, England

TITLE OF REPORT: N-Butyl Glycidyl Ether Acute Toxicity to Salmo Gairdneri

AUTHOR(S): R.R. Stephenson

REPORT ISSUED or STUDY COMPLETION DATE: 4-01-82

RECOGNIZED TEST METHOD: Although not reported as such, this study appears to be conducted in accordance with OECD Guideline No 203.

TEST TYPE: 96-h Acute toxicity (LC-50) to rainbow trout, Salmo gairdneri

GLP: Not reported

YEAR: 1982

SPECIES: Salmo gairdneri

TEST DETAILS: Healthy fingerlings were obtained from Itchen Valley Trout Farm, Alresford, Hampshire and acclimated to test conditions for ten days before use. Fish used in test were identified as Batch RT 14. Mean weight of 0.5 g and length between 3.3 and 4.0 cm. A 96-hour bioassay renewed daily with filtered (dechlorinated) tap water was conducted in 10-liter glass aquariums. Ten fish per test concentration were exposed. The tests were completed within a temperature range of 15+/-1°C. Five exposure concentrations ranging from 10-200 mg/l were used (10, 20, 50, 100, 200 mg/l). A sixth aquarium served as a control. Exposure duration was 96 hours.

STATISTICAL METHODS: The 96-h LC-50 was estimated using graphical interpolation on log/probit graph paper (APHA, 1975).

REMARKS: Analytical concentrations of test compound were not measured during course of test.

RESULTS: The 96-h LC-50 was estimated to be 65 mg/l.

DATA QUALITY: This study appears to be conducted in accordance with a recognized scientific procedure for determining adverse effects to fish. The study meets national and international scientific standards for performance of such methods and provides sufficient information to support the conclusions regarding toxicity to fish.

RELIABILITY:

1. w/o restriction [ ]
2. w restriction [x]
3. not reliable [ ]
4. not assignable [ ]

Reason: Actual exposure concentrations were not measured.

REFERENCES:

APHA. 1975. Standard Methods for the Examination of Water and Waste Water. 14th Edition, APHA, Washington.

Finney, D.J. 1971. Probit Analysis. Cambridge University Press.

TOXICITY TO AQUATIC PLANTS (ALGAE)

REPORT NUMBER: AL-1

STUDY TYPE: Algal acute toxicity test

TEST MATERIAL: n-butyl glycidyl ether; CAS # 2426-08-6; Supplied by: Shell Chemicals UK, Stanlow; Lot/Batch No: 696973; Solubility: Reported to be sufficiently soluble for all stock solutions to be prepared in distilled water.

STUDY NUMBER(S): SBGR.82.148

SPONSOR: SICC/CSAS; Shell Oil Company

TESTING FACILITY: Shell Toxicology Laboratory (Tunstall), Sittingbourne Research Center, Kent, England

TITLE OF REPORT: N-Butyl Glycidyl Ether Acute Toxicity to Salmo Gairdneri, Daphnia magna and Selenastrum capricornutum.

AUTHOR(S): R.R. Stephenson

REPORT ISSUED or STUDY COMPLETION DATE: 4-01-82

RECOGNIZED TEST METHOD: Reference protocol for the study was not reported although it appears to follow OECD No. 201.

TEST TYPE: Growth inhibition test; 4-day growth study

GLP: Not reported

YEAR: 1982

SPECIES: Selenastrum capricornutum, Green alga. S. capricornutum were taken from an axenic culture in STL derived from a strain ATCC 22662 obtained from the American Type Culture Collection, Maryland, USA.

ELEMENT BASIS: Four-day growth experiment. Eighteen flasks containing 50 ml of culture medium (Based on Miller and Green, 1978) were prepared. To 12 flasks were added test chemical to give log series of concentrations from 10-300 mg/l. Remaining 6 flasks served as controls. Each flask was inoculated to give an initial cell concentration of $5x10^3$ cells/ml. Flasks were incubated in a cooled orbital incubator under constant light at 24+/-1°C for 4 days.

EXPOSURE PERIOD: 96 hours

ANALYTICAL MONITORING: After 2 and 4 days, cell counts were made using Coulter Counter.

STATISTICAL METHODS: The mean relative growth rate (RGR) from each culture was calculated. The EC-50 value (concentrations causing 50% reduction in RGR compared to RGR of controls) was calculated by probit analysis using log transformed concentration values (Finney, 1971).

RESULTS: The 96-hour EC50 with respect to growth over the period, day 2 to day 4, was calculated to be 35 mg/l (95% confidence limits of 34-37 mg/l).

DATA QUALITY: This study appears to be conducted in accordance with a recognized scientific procedure for determining adverse effects to algae. The study meets national and international scientific standards for performance of such methods and provides sufficient information to support the conclusions regarding toxicity to algae.

RELIABILITY:

1. w/o restriction [x]
2. w restriction [ ]
3. not reliable [ ]
4. not assignable [ ]

REFERENCES:

APHA. 1975. Standard Methods for the Examination of Water and Waste Water. 14th Edition, APHA, Washington.

Finney, D.J. 1971. Probit Analysis. Cambridge University Press.

Miller, W.E. and Green, J.G. 1978. The Selenastrum capricornutum (Prinz) Algal assay bottle test. EPA-60/9-78-018.

ACUTE TOXICITY TO AQUATIC INVERTEBRATES

REPORT NUMBER: ADP-2

STUDY TYPE: Acute toxicity to Daphnia magna

TEST MATERIAL: n-butyl glycidyl ether; CAS # 2426-08-6; Solubility: Stock solution was prepared in distilled water.

STUDY NUMBER(S): SBGR.83.100

SPONSOR: SIRM, RS; Shell Oil Company

TESTING FACILITY: Shell Toxicology Laboratory (Tunstall), Sittingbourne Research Center, Kent, England

TITLE OF REPORT: Toxicity Tests with Daphnia magna: Acute Toxicity of Eight Test Materials to a Newly-Introduced Strain of D. magna in Reconstituted Fresh Water.

AUTHOR(S): B.M. Garforth

REPORT ISSUED or STUDY COMPLETION DATE: February 1983

TEST TYPE: Static acute toxicity, 48 hours

GLP: Not reported

YEAR: 1983

RECOGNIZED TEST METHOD: Study appears to be conducted in accordance with OECD Guideline No. 202, a 48-hour static toxicity test in Daphnia.

SPECIES: Daphnia magna

TEST DETAILS: A 48-hour static test was completed using Daphnia less than 24 hours old. Test medium (100 ml) was added to a series of 150-ml glass crystallizing dishes. Test medium was reconstituted fresh water. Test chemical was added in triplicate to dishes to give log series of concentrations ranging from 1 to 1000 mg/l. Ten Daphnia less than 24 hours old were added to each dish. Three dishes served as control. After 24 and 48 hours the number of immobilized D. magna were counted. Test temperature was 20+/-1°C.

STATISTICAL METHODS: The 24- and 48-hour EC-50s were calculated using probit analysis after log transformation of concentrations (Finney, 1971).

RESULTS: The 24-hour and 48-hour EC-50s were 22 mg/l and 3.9 mg/l (compared to original 24- and 48-hour EC-50 of 55 and 9.2 mg/l, respectively).

DATA QUALITY: Study conducted in accordance with national and international scientific methods and provides sufficient information to support the conclusions. This study is a repeat of SBGR.82.148 (1982), wherein the Daphnia used were often of poor condition, and rates of survival and reproduction were low. Further, it noted that not enough healthy animals were produced from that culture. Subsequently, a new culture was started from another source, using reconstituted fresh water (solution of inorganic salts in distilled water). The new culture was started in June 1982, with animals obtained from ICI Brixham Laboratory.

RELIABILITY:

1. w/o restriction	[x]
2. w restriction	[ ]
3. not reliable	[ ]
4. not assignable	[ ]

REFERENCES:

APHA. 1975. Standard Methods for the Examination of Water and Waste Water. 14th Edition, APHA, Washington.

Finney, D.J. 1971. Probit Analysis. Cambridge University Press.

Miller, W.E. and Green, J.G. 1978. The Selenastrum capricornutum (Prinz) Algal assay bottle test. EPA-60/9-78-018.

REPORT NUMBER: AINV-1

STUDY TYPE: Embryo larval test for Pacific Oyster

TEST MATERIAL: n-butyl glycidyl ether; CASRN # 02426-08-6; Solubility: Stock solution was prepared by weighing 2000 mg in 1000 ml of synthetic seawater.

STUDY NUMBER(S): SRC37992

SPONSOR: SIRM, RS; Shell Oil Company

TESTING FACILITY: Shell Research Limited, Sittingbourne Research Center

TITLE OF REPORT: Evaluation of the Pacific oyster (Crassostrea gigas) embryo larval test: Statistical validation of the test procedure and susceptibility to reference toxicants.

AUTHOR(S): Fairhurst, FM., Whale, GF., Gould, A., Walter, D.

REPORT ISSUED or STUDY COMPLETION DATE: January 1992

METHOD: Ability of embryos to develop into shelled D-stage veligers within 24 hours

RECOGNIZED TEST METHOD: Twenty-four-hour embryo developmental test; there presently is no such OECD method. However, EPA's harmonized test procedure, 850.1055 has a method similar to this, although EPA's method is a 48-hr method as opposed to a 24-hr method.

GLP: Not reported. However, test was being evaluated as part of an International Working Group established by the Paris Commission (PARCOM) to evaluate potential tests. The 24-hour embryo larval test using C. gigas was proposed by the Ministry of Agriculture, Fisheries and Food Fisheries Laboratory at Burnham-on-Crouch UK as a candidate for inclusion. In view of the increasing use of C. gigas embryo test both within SRC and internationally the test was statistically evaluated to ensure that the most suitable and cost effective design was used.

YEAR: 1992

SPECIES: Pacific oyster, Crassostrea gigas (Thunberg)

TEST DETAILS: Tests were carried out in artificial seawater (Tropic Marin-Aquatechnik, Wartenberg, Germany) made up in distilled water to 34 ppt. The oysters were conditioned to a pre-spawning state by maintaining in recirculating sea water at a temperature of 25°C and fed a mixed algal diet. Conditioning was conducted by a commercial oyster hatchery, Seasalter Shellfish Ltd., Whitstable, Kent. On arrival at the laboratory oysters were placed in aerated sea water at 20+/-2°C.
Detailed procedures were provided on embryo preparation, estimating egg and embryo density and fertilization of embryos. Exposure was completed by adjusting the density of a embryo suspension by dilution with sea water and inoculated into 30-ml glass bottles containing 30 ml of test media. Test concentrations ranged from 18-190 mg/l. Both exposure and control vessels

were run. Test vessels were incubated for 24 hours at 25°C with artificial lighting providing 16-h light and 8-h dark cycle.

STATISTICAL METHODS: The percent abnormality at each test concentration was calculated and Percent Net Response (PNR) calculated. The PNR was used to calculate a 24-hour EC-50 value using log transformed concentration values and the moving average angle method (EPA, 1985).

RESULTS: The 24 hour EC-50 was 74 mg/l.

DATA QUALITY: A high level of scientific quality and attention to detail was evident. Study was conducted in accordance with standard procedures for evaluating toxicity to bivalves, and provides sufficient information and documentation to support the conclusion.

RELIABILITY:

1. w/o restriction [x]
2. w restriction [ ]
3. not reliable [ ]
4. not assignable [ ]

HEALTH EFFECTS (TOXICITY) TESTS: ROBUST SUMMARIES

ACUTE TOXICITY

REPORT NUMBER: DS-4 (n)

STUDY TYPE: Skin Sensitization in Guinea Pigs

TEST MATERIAL: n-Butyl Glycidyl Ether; obtained from Fluka Chem. Corp., 96.8% pure, with 90 µg/ml epichlorohydrin content.

STUDY NUMBER(S): HET T2.2-173-003

SPONSOR: Dow Chemical USA

TESTING FACILITY: Mammalian and Environmental Toxicology Laboratory; Health & Environmental Sciences, U.S.A.; Dow Chemical USA; Midland, Michigan

TITLE OF REPORT: A Series of Guinea Pig Sensitization Tests for Structure-Activity Correlation - Epoxides

AUTHOR(S): J.E. Betso, R.E. Carreon, P.W. Langvardt and E. Martin

REPORT ISSUED or STUDY COMPLETION DATE: May 14, 1986

RECOGNIZED METHOD, i.e. OECD: Modified test method of Maguire, one of several recognized and acceptable test methods for determining the contact sensitization of test substances; OECD 406.

GLP: Not stated

SPECIES/SEX: Healthy adult male Hartley albino guinea pigs; weighing 225-300 g; source was Charles River Breeding Laboratories, Inc., Kingston, NY.

DOSE LEVEL(S) and NUMBER OF DOSES: 10.0% solution in DOWANOL DPM glycol ether/Tween 80 surfactant (9:1).

NUMBER OF ANIMALS/DOSE: Ten test and 10 control guinea pigs.

MEASURED ENDPOINT/INDEX (i.e. Sensitization): Test material did not induce delayed contact hypersensitivity in guinea pigs.

STUDY METHOD: Tested in accordance with the Maguire Split Adjuvant technique (1972) (one of several sensitization methods acceptable to OECD and EPA). Ten animals were allocated to the test group and 10 to a positive control group. Prior to the induction phase, material was tested to determine the non-irritating concentration. Approximately 48 hours before application, the backs of the guinea pigs were clipped with electric clippers. The following day, NEET, a hair depilatator was applied. Twenty-four hours later, 10 guinea pigs received 4 applications of the undilute test material within 10 days during the insult phase. An additional group of 10 guinea pigs received the positive control (DER 331 epoxy resin as a 10% solution).

Induction phase: Each insult application consisted of 0.1 ml of test material or the positive control applied to a 15mm x 15mm gauze patch, attached to the animal's back, and occluded. The first patch was removed after 48 hours and a second patch applied. At the time of the third application, a total of 0.2 ml of Freund's Adjuvant was injected i.d. at multiple points adjacent to the insult site. Forty-eight hours later the patch was removed and a fresh patch w/ 0.1 ml of test material was applied. Twenty-four hours later this patch was removed. Skin reactions were scored each time the patch was removed.

Challenge phase: Two weeks after the last exposure all test and control animals are challenged with 0.1 ml test material and solvent but not covered with patches. Sites were scored again at 24 and 48 hours.

RESULTS/OBSERVATIONS: There was no positive skin sensitization reaction.

DATA QUALITY: Study appeared to follow a modified protocol of Maguire, an acceptable test method for dermal sensitization. It was not clear that this study was conducted under GLP regulations. Individual animal scores were not reported, the method of scoring the intensity of each reading was not reported and it is not clear that a solvent control was used. However, the test was run concurrently with several other epoxy resins and each gave different and positive responses,

confirming the sensitivity of the test method. Thus, negative results reported for this study can be supported by the study report.

RELIABILITY:

1 w/o restriction	[ ]
2 w restriction	[X]
3 not reliable	[ ]
4 not assignable	[ ]

Reason: Followed the Split Adjuvant protocol of Maguire, an acceptable test method for dermal sensitization. It was not clear that this study was conducted under GLP regulations. Individual animal scores were not reported.

GENETIC TOXICITY

TOXICITY REPORT NUMBER: MU-7 (n)

STUDY TYPE: In Vivo Dominant Lethal Assay in Mice

TEST MATERIAL: n-Butyl Glycidyl Ether; supplied by Shell-Petroleum; 95% pure

STUDY NUMBER(S): Published report in Mutation Research, 124: 225-233 (1983)

SPONSOR: Not stated

REPORT ISSUED or STUDY COMPLETION DATE: Published in Mutation Research, 124: 225-233 (1983)

TESTING FACILITY: Department of Preventive Medicine and Community Health and Department of Pathology, University of Texas Medical Branch, Galveston, TX 77550

TITLE OF REPORT: Dominant Lethal Effects of n-butyl glycidyl ether in Mice.

AUTHOR(S): E.B. Whorton, T.G. Pullin, A.F. Frost, A. Onofre, M.S. Legator and D. S. Folse

REPORT ISSUED or STUDY COMPLETION DATE: 1983

RECOGNIZED METHOD, i.e. OECD: Consistent with OECD Guideline 478.

GLP: Not stated

TEST ANIMALS USED: BDF hybrid mice; males were 8-10 weeks old at commencement of experiment, and females were 8-10 weeks old when mated.

TEST COMPOUND CONCENTRATIONS USED: 0, 0.375, 0.75, and 1.5 g/kg.

ROUTE OF ADMINISTRATION: Applied topically to shaved dorsal areas.

CONTROL MATERIALS: Solvent control was 0.9% saline. No positive control was used because TEM had been previously tested in an earlier experiment in the same lab (fetal death rate: 52.8%).

TEST PERFORMANCE: Each male mouse was mated with 3 virgin females 2 weeks prior to administration of test material to determine mating efficiency. Males were treated with the test agent 3 times per week for a total of 8 weeks. Each male was weighed weekly and dosage adjusted based upon mean weights. Following the last treatment, each male was mated with 3 virgin females per week for 3 weeks. All females were sacrificed from the mid-week of caging (no check for vaginal plug), and examined for total implants and fetal deaths. Following the final mating, each male was sacrificed and the testes removed and histologically examined.

STATISTICAL ANALYSIS: ANOVA procedures used as described by Whorton (1981). Three variables were used to evaluate the effects: Pregnancy Rate, Fetal Death Rate, and Number of Implants per Pregnant Female. The averaged angular transformation described by Freeman and Tukey (1950) was applied prior to analysis.

REPORT RESULTS: There was no effect on Pregnancy Rate or Number of Implants in either experiment 1 or 2. There was a significant increase in the Fetal Death Rate at the highest dose in experiment 1, but it could not be confirmed due to a similarly high rate in controls in experiment 2. Analysis of testes failed to demonstrate any compound related effects on sperm viability or morphology.

CONCLUSION: n-Butyl glycidyl ether produced equivocal dominant lethal effects in this assay, at the high dose of 1.5 g/kg.

DATA QUALITY: At the time this particular test was conducted there was no recognized international standard for such assays. There is now an OECD Test Guideline 478 (approved in 1984). It is further recognized that M. Legator was a lead scientist in the development of the dominant lethal assay and his work has been published, peer-reviewed and referenced in OECD Guidelines. Shortcomings in this study design (e.g. C.L. not counted; and equivocal results) are not considered major protocol deficiencies due to the available information from all previous tests.

RELIABILITY:

1 w/o restriction	[ ]
2 w restriction	[X]
3 not reliable	[ ]
4 not assignable	[ ]

REPORT NUMBER: MU-13 (n)

STUDY TYPE: Salmonella/Mammalian Microsome Assay

TEST MATERIAL: n-Butyl Glycidyl Ether; purified by vacuum distillation; 99.1% pure; 0.4% alcohol; 12.4% oxirane oxygen

STUDY NUMBER(S): Published report by Proctor and Gamble in Mutation Research, 90: 213-231 (1981)

SPONSOR: Proctor and Gamble Co.

TESTING FACILITY: Miami Valley Laboratories, Cincinnati, OH; Hazleton Laboratories of America, Vienna, VA; and SRI International, Menlo Park, CA

TITLE OF REPORT: Mutagenicity of alkyl glycidyl ethers in three short-term assays.

AUTHOR(S): E.D. Thompson; W.J. Coppinger; C.E. Piper; N. McCarroll; T.J. Oberly; and D. Robinson

REPORT ISSUED or STUDY COMPLETION DATE: June 5, 1981 (published)

RECOGNIZED METHOD, i.e. OECD: Predates OECD Guideline 471; followed the method of Ames et al. (1975).

GLP: Not stated; but probably did follow GLP considering the labs involved

TEST ORGANISM USED: Salmonella typhimurium strains: TA98, TA100, TA1535, TA1537, and TA1538; obtained from Dr. B. N. Ames.

TEST COMPOUND CONCENTRATIONS USED: Six concentrations were evaluated in triplicate: 8.2, 24.7, 74, 222.2, 666.7 and 2000 µg/plate.

CONTROL MATERIALS: The control materials employed were as follows:

Metabolic activation (S9):

2-Aminoanthracene (5 µg/plate): with all strains

No Metabolic activation:

N-Methyl-N'-nitro-N-nitrosoguanidine (5 µg/plate): TA100 and TA1535
2-Nitrofluorene (50 µg/plate): TA1538 and TA98
9-Aminoacridine (125 µg/plate): TA1537

Vehicle Control:

DMSO (0.1 ml)
Water

ACTIVATION: Derivation of S9 fraction not fully described; Aroclor 1254-induced rat liver from Sprague-Dawley rats. Sex of rats not specified.

TEST PERFORMANCE:

o Type of Salmonella Assay	_X_	Standard plate assay
	____	Pre-incubation (overnight)
	____	"Prival" modification
	____	Spot Test

PROTOCOL: Test report noted that the study was conducted in accordance with the procedures published by Ames *et. al* (1975). A preliminary cytotoxicity test was conducted to determine the appropriate dose levels to use in the mutagenicity assay. Dilutions were prepared in DMSO and a single plate prepared according to Ames (1975). After 48 hours incubation at 37° C, the number of revertant colonies was determined, and the highest dose was the concentration that reduced the background lawn 50-75%, compared to solvent control. Based upon these results 6 concentrations were evaluated in triplicate.

REPORT RESULTS:

o Preliminary Cytotoxicity assay: Doses used were not reported.

o Mutation assay: Test compound did not induce any mutations in TA1537, TA1538 or TA98. Test material was mutagenic in TA100 and TA1535 w/ and w/o metabolic activation; although it was more reactive in TA1535, with effects noted at the lowest dose w/o activation.

CONCLUSION: Test material was mutagenic in S. typhimurium TA1535 and TA100 both w/ and w/o activation.

DATA QUALITY: The study was conducted in accordance with a recognized international scientific procedure and followed the test protocol and procedures of Ames (1975). Complete study results were presented supporting the conclusions that n-butyl glycidyl ether was mutagenic in this test system. Full description of the test material was provided

RELIABILITY:

1 w/o restriction	[ ]
2 w restriction	[X]
3 not reliable	[ ]
4 not assignable	[ ]

Reason: Although the procedures themselves were not fully elaborated in the study, the report noted that it followed the procedures of Ames *et. al.* (1975), the recognized reference

method for this assay. Further, the data and study results were fully presented and complete, and the test material was 99.1% pure.

REPORT NUMBER: MU-14 (n)

STUDY TYPE: Mouse Lymphoma Assay (In Vitro)

TEST MATERIAL: n-Butyl Glycidyl Ether; purified by vacuum distillation; 99.1% pure; 0.4% alcohol; 12.4% oxirane oxygen

STUDY NUMBER(S): Published report by Proctor and Gamble in Mutation Research, 90: 213-231 (1981)

SPONSOR: Proctor and Gamble Co.

TESTING FACILITY: Miami Valley Laboratories, Cincinnati, OH; Hazleton Laboratories of America, Vienna, VA; and SRI International, Menlo Park, CA

TITLE OF REPORT: Mutagenicity of alkyl glycidyl ethers in three short-term assays.

AUTHOR(S): E.D. Thompson; W.J. Coppinger; C.E. Piper; N. McCarroll; T.J. Oberly; and D. Robinson

REPORT ISSUED or STUDY COMPLETION DATE: June 5, 1981 (published)

RECOGNIZED METHOD, i.e. OECD: Predates OECD Guideline 476; followed the method of Clive and Spector (1975 and 1979).

GLP: Not stated; but probably did follow GLP considering the labs involved

TEST CELLS USED: Mouse lymphoma L5178Y heterozygous TK+/- (obtained from Dr. D. Clive)
Properly maintained: NS
Periodically checked for mycoplasm: NS
Periodically checked for karyotype stability: NS
Periodically "cleansed" against high spontaneous background of TK-/- cells: NS

LOCUS EXAMINED: thymidine kinase (TK), using TFT (trifluorothymidine, 1 µg/ml)

TEST COMPOUND CONCENTRATIONS USED:

Cytotoxicity assay: with and w/o activation at several dose levels (a 1000-fold range); each concentration tested w and w/o activation, solvent control (corn oil), and three positive controls [EMS used w/o S9 activation; dimethyl nitrosamine with uninduced rat liver S9; and 2-AAF w/ rat liver S9].

Forward Mutation Assay: after a 2-day expression period, approximately 12 concentations, ranging from 84- 800 μg/ml were selected, based on the degree of toxicity.

CONTROL MATERIALS:

Ethyl Methanesulfonate (EMS)
2-Acetylaminofluorene (AAF)
Dimethylnitrosamine (DMN)

Solvent Control: Corn oil

Positive Control:

Metabolic Non-activation:
EMS: 620 μg/ml
Uninduced Rat Liver S9:
DMN: 74 μg/ml
Induced Rat Liver S9:
2-AAF: 100 μg/ml

ACTIVATION: Aroclor 1254-induced rat liver S9 fraction was prepared from Sprague-Dawley rats. Non-induced liver S9 was prepared in the same manner only with corn oil instead of Aroclor 1254. Prepared fresh each day.

TEST PERFORMANCE:
Cell Treatment: 6 million actively growing L5178Y cells are cleansed as described by Clive et al. to reduce the frequency of TK-/- cells; placed in 6 ml $F^{10}P$ medium, 0.1 ml test material, and either 4 ml S9 or 4 ml $F^{10}P$ added. Tubes were gassed with 5% CO_2 and incubated at 37°C in a roller drum for 4 hours. The cells are centrifuged (500 x g for 10 minutes), washed with $F^{10}P$, resuspended in $F^{10}P$, gassed with 5% CO_2 and incubated at 37° C. At 24 and 48 hours, the total cell number and viability are determined in the presence of tryphan blue in a hemocytometer. After the initial 24 hours, the viable cell count was readjusted to 3 X 10^5 cells/ml. After the 48-hr incubation period, cells were resuspended in $F^{10}P$ at 1 x 10^6 viable cells/ml determined by tryphan blue exclusion. A portion of the cells were diluted and 1 ml plated onto non-selective medium for viable cell counts (approx. 100 cels/plate); 0.5 ml of the original cell suspension (0.5 x 10^6 cells) was plated in the presence of TFT (1 μg/ml), incubated for 10-12 days, and the number of mutant colonies and viable colonies were determined.

REPORT RESULTS: The results demonstrated that the n-butyl glycidyl ether was positive in all three assays: w/o metabolic activation, w/ metabolic activation by non-induced S9, and w/ induced S9. The positive controls gave the expected response and confirmed the sensitivity of the test assay.

CONCLUSION: n-Butyl glycidyl ether was mutagenic in the test system w/ and w/o S9 activation. It was more reactive in the test system w/o metabolic activation.

DATA QUALITY: Study appeared to follow recognized published scientific procedure for determining the adverse effects of a test substance in an In Vitro Mutagenicity Assay (Mouse Lymphoma Test); presumably following GLP regulations (although not stated). It was not stated how the cells were maintained or whether they were checked for mycoplasm. Positive controls used were those recognized and required in contemporary mutagenic assays, and confirmed the sensitivity of the test procedure to reveal mutagenic responses in the test system. The study appears to meet national and international scientific standards and provides sufficient information to support the conclusions regarding the mutagenic potential demonstrated from the study data.

RELIABILITY:

1 w/o restriction	[ ]
2 w restriction	[X]
3 not reliable	[ ]
4 not assignable	[ ]

Reason: Although the method did not completely describe all of the procedures followed, it was sufficiently detailed and provided minimum scientific standards to support the conclusions presented.

REPORT NUMBER: MU-15 (n)

STUDY TYPE: UDS DNA synthesis (In Vitro)

TEST MATERIAL: n-Butyl Glycidyl Ether; purified by vacuum distillation; 99.1% pure; 0.4% alcohol; 12.4% oxirane oxygen

STUDY NUMBER(S): Published report by Proctor and Gamble in Mutation Research, 90: 213-231 (1981)

SPONSOR: Proctor and Gamble Co.

TESTING FACILITY: Miami Valley Laboratories, Cincinnati, OH; Hazleton Laboratories of America, Vienna, VA; and SRI International, Menlo Park, CA

TITLE OF REPORT: Mutagenicity of alkyl glycidyl ethers in three short-term assays.

AUTHOR(S): E.D. Thompson; W.J. Coppinger; C.E. Piper; N. McCarroll; T.J. Oberly; and D. Robinson

REPORT ISSUED or STUDY COMPLETION DATE: June 5, 1981 (published)

RECOGNIZED METHOD, i.e. OECD: Predates OECD Guideline 482; followed the method of San and Stich (1975) and Rasmussen and Painter (1966).

GLP: Not stated; but probably did follow GLP considering the labs involved

TEST CELLS USED: WI38 obtained from the American Type Culture Collection. (human cell line).

LOCUS EXAMINED: Increased DNA repair determined by monitoring [^{3}H] thymidine incorporation into DNA of G_0 phase cells exposed to test material.

TEST COMPOUND CONCENTRATIONS USED:

Cytotoxicity assay: with and w/o activation at several dose levels to determine the maximal testable level. Each concentration tested w and w/o activation, solvent control (DMSO), and two positive controls [4-NQO used w/o S9 activation; dimethyl nitrosamine with S9 activation].

Doses selected: 0.24, 0.36, 0.53, 0.8 and 1.2 µl/ml (w/o S9 activation); and 0.5, 1.0, 2.0, 4.0 and 8.0 µl/ml (w/ S9 activation).

CONTROL MATERIALS:

4-Nitroquinoline-N-oxide (4-NQO)
Dimethylnitrosamine (DMN)

Solvent Control: DMSO

Positive Control:

Metabolic Non-activation:
4-NQO: 10^{-5} M
Metabolic Activation;
DMN: 5×10^{-2} M

ACTIVATION: 9000 x g supernatant (S9) of liver homogenate from adult male Swiss-Webster mice. Co-factors added to S9 included: nicotinamide, G-6-P, $MgCl_2$-$6H_2O$ and NADP.

TEST PERFORMANCE:

Cell Treatment:

a. WI38 cells grown in T25 tissue culture flasks were initiated in Eagle's Basal Medium containing 10% fetal bovine serum. Cells grown to confluency and maintained in medium containing 0.5% serum for 5-6 days preceding UDS assays in order to produce synchronous cultures of contact inhibiting cells in G_0 mitotic phase. Cultures were incubated for 1 hour with 10^{-2} M hydroxyurea (HU) before each assay.

UDS assay:

a. WI38 incubated with test material in DMSO (<1%) at 37° C in 1 µCi/ml of ^{3}H-TdR. Testing w/o activation: cells exposed simultaneously to test compound, and to ^{3}H-TdR for 3 hours.

Testing w/ activation: cells incubated with test compound, ^{3}H-TdR, and S9 for 1 hour, then only ^{3}H-TdR for an additional 3 hours.

b. A modification of Schmidt and Thannhauser (1945) was used to extract DNA from the cells; 0.1 ml of the DNA solution was used to measure DNA content after reacting with diphenylamine and a second aliquot used to determine incorporation of ^{3}H-TdR by scintillation counting. Results are the average of 6 replicate samples.

REPORT RESULTS: The results demonstrated that the n-butyl glycidyl ether was not mutagenic in this test system. The positive controls gave the expected response and confirmed the sensitivity of the test assay.

CONCLUSION: n-Butyl glycidyl ether was not mutagenic in the test system w/ and w/o S9 activation.

DATA QUALITY: Study appeared to follow recognized published scientific procedure for determining the adverse effects of a test substance in an in vitro UDS assay employing WI38 cells; presumably following GLP regulations (although not stated). Positive controls used were those recognized and required in contemporary mutagenic assays, and confirmed the sensitivity of the test procedure to reveal mutagenic responses in the test system. The study appears to meet minimum national and international scientific standards and provides sufficient information to support the conclusions regarding the mutagenic potential demonstrated from the study data.

RELIABILITY:

1 w/o restriction	[ ]
2 w restriction	[X]
3 not reliable	[ ]
4 not assignable	[ ]

Reason: Although the method did not completely describe all of the procedures followed, it was sufficiently detailed and provided minimum scientific information to support the conclusions presented.

HEALTH EFFECTS (TOXICITY) TESTS: ROBUST SUMMARIES

ACUTE TOXICITY

REPORT NUMBER: AO-1 (t)

STUDY TYPE: Acute Oral Toxicity

TEST MATERIAL: Tertiary Butyl Glycidyl Ether; clear colorless liquid; 99+% pure

STUDY NUMBER(S): T36.1-59376-2

SPONSOR: Dow Chemical USA

TESTING FACILITY: Chemical Biology Research, Dow Chemical Company, Midland, MI

TITLE OF REPORT: Toxicological Properties and Industrial Handling Hazards of 1-Tertiary-Butoxy-2, 3-epoxy Propane

AUTHOR(S): L.W. Rampy

REPORT ISSUED or STUDY COMPLETION DATE: March 7, 1972

RECOGNIZED METHOD, i.e. OECD: Regulation for the Enforcement of the Federal Hazardous Substances Act (FR, September 17, 1964); and NAS Publication 1138, 1977.

GLP: Pre-GLP, but appeared to follow good record keeping practices

SPECIES/SEX: Rats, males weighing 267-305 grams; strain not specified.

DOSE LEVEL(S) and NUMBER OF DOSES: 0.126, 0.252, 0.5, 1.0 and 2.0 gm/kg of undiluted test material.

NUMBER OF ANIMALS/DOSE: Two rats per dose.

MEASURED ENDPOINT/INDEX (i.e. LD50, PII): Acute toxicity, body weight changes, and mortality. One rat died in the highest dose, 2.0 gm/kg.

STUDY METHOD: Rats were fed a single dose of undiluted test material via oral intubation; animals fasted overnight prior to dosing. All animals were weighed prior to dosing and at weekly intervals until study termination 2 weeks post-dosing. One rat per dose was subjected to gross necropsy.

RESULTS/OBSERVATIONS: There were no adverse effects observed, no effect on body weight gain, no gross pathological changes, and no mortality at the lowest 3 dose levels; at 1 gm/kg there was slight accumulation of darkened material around the external nares and the mucosal surface of

the stomach was slightly edematous; at 2.0 gm/kg one rat died, and there was darkened material around the external nares and the mucosal surface of the stomach was edematous and congested.

DATA QUALITY: Study appeared to follow minimum testing standards for acute oral toxicity testing, even though the number of rats was limited and only males were tested. The test material was 99% pure and the lab notes provide minimum but sufficient details to assess the acute oral hazards from the test substance.

RELIABILITY:

1 w/o restriction	[ ]
2 w restriction	[X]
3 not reliable	[ ]
4 not assignable	[ ]

Reason: Followed minimum standards for conducting acute toxicology in experimental animals. Results have been extracted from the lab report and lab notes.

REPEATED DOSE (Subchronic) TOXICITY

REPORT NUMBER: SC-1 (t)

STUDY TYPE: 2-Week Inhalation Toxicity

TEST MATERIAL: Tertiary Butyl Glycidyl Ether; clear colorless liquid; density: 0.909 g/ml; purity; 98.8%; B.P. 152° C (760 mm Hg)

STUDY NUMBER(S): K-59376-(6)

SPONSOR: Dow Chemical USA

TESTING FACILITY: Toxicology Research Laboratory, Health & Environmental Sciences, USA, Dow Chemical USA, Midland, MI

TITLE OF REPORT: t-Butyl Glycidyl Ether (TBGE): A 2-Week Inhalation Study in Rabbits, Rats, and Mice

AUTHOR(S): T.S. Gushow and J.F. Quast

REPORT ISSUED or STUDY COMPLETION DATE: March 20, 1984

RECOGNIZED METHOD, i.e. OECD: Appears to follow OECD 412 for 14-Day Inhalation Toxicity.

GLP: Yes

SPECIES/SEX: Male and female Fischer 344 rats and $B_6C_3F_1$ mice from Charles River Breeding Laboratories; and male and female New Zealand white rabbits from Langshaw Farms, Augusta, MI.

AGE at Start of Test: Rabbits: 5 1/2 months; Rats: 8 weeks; Mice: 14 weeks.

ROUTE: Inhalation

DURATION OF TEST: 14 days (10 exposures); doses were administered 6 hours per day, 5 days/week.

DOSE LEVEL(S) and NUMBER OF DOSES: All animals were exposed to 0, 100, 300 or 1000 ppm (equivalent to 0, 0.52, 1.56, and 5.2 mg/L). Test material vapor was generated by metering it into vaporization tubes at a controlled rate, and then feeding it into the chamber inlet ducts with compressed air, which was preheated (55-85°C). Test material concentration was analyzed once per hour during exposure via GC and flame ionization. The nominal concentration was calculated for each chamber, each exposure day. A standard curve was run prior to the first exposure to check the analytical system. Exposures were conducted in 4.1 m^3 stainless steel and glass chambers under dynamic air conditions. Flow rate and air exchange rate not reported.

NUMBER OF ANIMALS/DOSE: Rats: 5M/5F per dose; Rabbits: 2M/2F per dose; and Mice: 6M/6F per dose.

VEHICLE: None

BODY WEIGHT MEASUREMENTS: Determined individually throughout the 10 days prior to exposure period. Mean body weight values for all test animals (both sexes) in 300 (except mice at 300) and 1000 ppm groups were significantly lower than those of control, and were dose related. No body weight decrement was evident at 100 ppm.

FOOD CONSUMPTION/FOOD EFFICIENCY: Not stated.

HEMATOLOGY: Rats: 7th exposure day - tail vein blood samples; Rabbits: 6th exposure day - ear vein; Mice: at necropsy. Hematological measurements included: RBC, WBC, differential counts, PCV, and Hgb concentration. There were no effects at 100 and 300 ppm. At 1000 ppm values were not considered interpretive due to debilitated condition of all animals.

CLINICAL CHEMISTRY: Rats: 7th exposure day - tail vein blood samples; Rabbits: 6th exposure day - ear vein; Mice: at necropsy. Clinical chemistry measurements included: BUN, SGPT, AP, and glucose. There were no effects at 100 and 300 ppm. At 1000 ppm values were not considered interpretive due to debilitated condition of all animals.

URINALYSIS: Obtained in rats only; no adverse effects observed.

STATISTICAL METHODS: Hematology data, specific gravity of urine, clinical chemistry data, body weights, organ weights and organ-to-body weight values were evaluated by ANOVA and Dunnett's test, level of significance $p < 0.05$.

ORGAN WEIGHTS: Absolute and relative organ weights (organ-body wt) were measured for liver, kidney, brain, heart, thymus and testes. Most noticeable effect was decreased liver weights at 300 ppm and greater. Due to the significant body weight decrease at the same dose, the organ-body-weight changes are a reflection of the debilitated condition of animals at this dose level.

GROSS PATHOLOGY: Full compliment of tissues were fixed and examined. Treatment related effects occurred at 300 ppm and 1000 ppm in all species. Rabbits: decreased body fat and fecal material in G.I. tract. Rats: decreases in body fat, thymic size, lymphoid organs, abdominal and thoracic viscera, also corneal cloudiness and evidence of pneumonia. Mice: decreases in body fat, thymic size and lymphoid organs.

HISTOPATHOLOGY: Tissue preserved but not performed.

CLINICAL OBSERVATIONS: Rabbits: 300 ppm - exudative rhinitis, lethargy; death in all rabbits at 1000 ppm. Rats: 300 ppm and above - lethargy. Mice: 300 ppm - lethargy, postural and gait changes; 4/6 females died.

FINDINGS/MEASURED ENDPOINT/INDEX (i.e. LOEL, NOEL): A NOAEL was demonstrated at 100 ppm based upon body weight changes and debilitated condition at 300 ppm and above.

DATA QUALITY: Study was conducted in accordance with a recognized scientific procedure for evaluating the inhalation toxicity to a test substance for 14-days in experimental animals. Study was conducted in compliance with GLP regulations and provides sufficient information to support the NOAEL demonstrated in the study.

RELIABILITY:

1 w/o restriction	[X]
2 w restriction	[ ]
3 not reliable	[ ]
4 not assignable	[ ]

Reason: Followed recognized toxicology testing procedures and test material adequately characterized.

REPORT NUMBER: SC-2 (t)

STUDY TYPE: 90-Day Inhalation Toxicity Study

TEST MATERIAL: Tertiary Butyl Glycidyl Ether; clear colorless liquid; density: 0.909 gms/ml; purity; 99%; B.P. 152° C (760 mm Hg); vapor pressure 3.2 mm Hg @25°C.

STUDY NUMBER(S): K-59376-(7)

SPONSOR: Dow Chemical USA

TESTING FACILITY: Toxicology Research Laboratory, Health & Environmental Sciences, USA, Dow Chemical USA, Midland, MI

TITLE OF REPORT: t-Butyl Glycidyl Ether (TGBE): 90-Day Inhalation Study in Rats, Mice and Rabbits.

AUTHOR(S): R.R. Miller and J.F. Quast

REPORT ISSUED or STUDY COMPLETION DATE: June 6, 1984

RECOGNIZED METHOD, i.e. OECD; EPA: Follows OECD 413 for 90-Day exposure period.

GLP: Yes

SPECIES/SEX: Male and female Fischer 344 rats and $B_6C_3F_1$ mice from Charles River Breeding Laboratories; and male and female New Zealand white rabbits from Langshaw Farms, Augusta, MI.

AGE at Start of Test: Rabbits: 7-8 months; Rats: 7-9 weeks; Mice: 7-9 weeks.

ROUTE: Inhalation

DURATION OF TEST: 90 days; doses were administered 6 hours per day, 5 days/week for 13 weeks.

DOSE LEVEL(S) and NUMBER OF DOSES: 0, 25, 75 and 225 ppm (equivalent to 0, 0.13, 0.4 and 1.19 mg/L). The vapor was generated by bubbling dry air through the test material in gas-washing bottles. The amount of material vaporized was controlled by adjusting the flow of air through the gas-washing bottle. The resulting vapor was then fed into a port on the chamber air intake line and diluted to the exposure concentration with room air. The stainless steel and glass exposure chambers used had a total volume of 4.3 cubic meters. Dynamic airflow rate of 800 liters/minute.

NUMBER OF ANIMALS/DOSE: Rats: 10M/10F per dose; Rabbits: 4M/4F per dose; and Mice: 10M/10F per dose.

VEHICLE: None.

BODY WEIGHT MEASUREMENTS: Determined individually immediately prior to first exposure and weekly thereafter. There were significant decreased body weight gain in rats, mice and rabbits at 225 ppm, with equivocal decreases in rats and mice at 75 ppm.

FOOD CONSUMPTION/FOOD EFFICIENCY: Not stated.

CAGESIDE OBSERVATIONS: There were no mortalities; nasal exudation was noted only in male and female rabbits at 225 ppm. All other species were unremarkable throughout the study.

HEMATOLOGY: Determined in all Rats: week 12 - orbital sinus blood samples; all Rabbits: week 12 - ear vein; all Mice: at necropsy. Hematological measurements included: RBC, WBC, differential counts, PCV, and Hgb concentration. There were no effects in rats and mice at any dose. Blood samples and bone marrow cytology of rabbits confirmed no adverse effects at any dose in this species as well.

CLINICAL CHEMISTRY: Blood samples for all species taken at necropsy. Clinical chemistry measurements included: BUN, SGPT, AP, and glucose. There were no adverse effects at any dose in any species.

URINALYSIS: Obtained in rats only; no adverse effects observed. The following parameters were determined: specific gravity, pH, protein, glucose, ketones, bilirubin, blood and urobilinogen.

STATISTICAL METHODS: Body weight, hematology, clinical chemistry, organ weight, organ/body weight, and organ/brain weight values were statistically evaluated by ANOVA and Dunnett's test. Variances of group mean body weights analyzed by Bartlett's test $p<0.05$.

ORGAN WEIGHTS: Absolute and relative organ weights (organ-body wt) were measured for liver, kidney, brain, heart, thymus and testes. Most noticeable effect was decreased absolute liver weights at 75 and 225 ppm in female rats and at 25, 75 and 225 ppm in female mice. Authors state that changes in organ weights were a result of decreases in body weight. Decreased body weight was demonstrated in female rats at 75 and 225 ppm. However, no significant decrease body weight gain was demonstrated in female mice at 25 and 75 ppm, where absolute liver weight was also significantly decreased. Thus, the statement by the authors must be clarified.

GROSS PATHOLOGY: Full compliment of tissues were fixed and examined. Treatment related effects occurred as follows: Rats: decreases in body fat, thymic size, and also corneal cloudiness at 225 ppm (both sexes). Mice: decreases in body fat, thymic size and nasal discharge at 225 ppm (both sexes). Rabbits: decreased body fat, thymic size at 225 ppm, and atelectasis of lung at 75 and 225 ppm (males); females showed the same body fat and thymic size changes at 225 ppm, but darkened areas of lung and atelectasis were evident in 1/4 at 25 ppm, 2/4 at 75 ppm, and 3/4 at 225 ppm, suggesting a dose related effect. In addition, the following reproductive tissues/organs were examined grossly in the females of all three species: ovaries, oviducts, uterine horn, cervix and vagina. In male rats and mice the reproductive tissues/organs included: testes, epididymides, prostate, seminal vesicles, coagulating gland and preputial gland (rats only). In male rabbits only the testes, epididymides and accessory sex glands were examined. No adverse effects were observed in any of these organs/tissues examined.

HISTOPATHOLOGY: A full compliment of tissues were fixed and examined in all species histologically. Rats: Males - loss of cells/thickening or flattening of olfactory epithelium in nasal turbinates at 75 and 225 ppm, with hyperplasia at 225 ppm; decrease size of hepatocytes in liver at

225 ppm; decrease in thymocytes at 225 ppm. Females - increased subepithelial inflammatory cell infiltrate in nasal turbinates at 75 and 225 ppm, generalized thickening (hyperplasia) in respiratory epithelium at 225 ppm, and loss of cells, thickening or flattening of olfactory epithelium at 225 ppm; accentuated lobular pattern or decrease in size of hepatocytes at 75 and 225 ppm; decrease in thymocytes at 225 ppm. Mice: Males - increased subepithelial inflammatory cell infiltrate in nasal turbinates at 75 and 225 ppm, thickening (hyperplasia) in respiratory epithelium at 75 and 225 ppm, and loss of cells, thickening or flattening of olfactory epithelium at 75 and 225 ppm; decreased size of hepatocytes at 25, 75 and 225 ppm. Females - increased subepithelial inflammatory cell infiltrate in nasal turbinates at 75 and 225 ppm, thickening (hyperplasia) in respiratory epithelium at 75 and 225 ppm, and loss of cells, thickening or flattening of olfactory epithelium at 75 and 225 ppm; decreased size of hepatocytes at 25, 75 and 225 ppm. Rabbits: Males - increase in exudative rhinitis at 225 ppm; focal subacute pneumonitis at 75 and 225 ppm; increased thickness or hyperplasia of tracheal epithelium at 75 and 225 ppm; decreased size of hepatocytes at 25, 75 and 225 ppm. Females - suppurative bronchopneumonia at 25, 75 and 225 ppm; increased thickness or hyperplasia of tracheal epithelium at 75 and 225 ppm. Further, as noted above under Gross Pathology, the following reproductive tissues/organs were examined histologically in the females of all three species: ovaries, oviducts, uterine horn, cervix and vagina. In male rats and mice the reproductive tissues/organs included: testes, epididymides, prostate, seminal vesicles, coagulating gland and preputial gland (rats only). In male rabbits only the testes, epididymides and accessory sex glands were examined. No adverse effects were observed in any of these organs/tissues when examined microscopically.

FINDINGS/MEASURED ENDPOINT/INDEX (i.e. LOAEL, NOAEL): Authors state that a NOAEL was demonstrated at 25 ppm. For rats, a NOAEL of 25 ppm was demonstrated in both sexes. In mice, however, examination of the data reveals a decrease size of hepatocytes at all dose levels in both sexes; also, in male rabbits at all doses. Interestingly, in female rabbits there was also decreased hepatocyte size in control as well as all dose groups, but no associated body weight decrease. Decrease glycogen content in liver hepatocytes, in the absence of any other adverse effects in the liver pathology, is more a reflection of nutritional status than of a chemically induced effect. Food consumption and food efficiency data (not available) would be useful information to help clarify such a finding. However, in view of the lack of adverse effects in hematology, clinical chemistry, urinalysis, and all other gross and histologic determinations at 25 ppm, it can be concluded that a NOAEL was demonstrated at 25 ppm in all rats, mice and rabbits. Further, there were no adverse effects observed in the reproductive organs/tissues in rats, mice, and rabbits at doses up to and including 225 ppm.

DATA QUALITY: Study was conducted in accordance with recognized national and international scientific procedures for determining the adverse effects of a test substance via inhalation when administered repeatedly for 13 weeks in experimental animals. Study was conducted in compliance with GLP regulations, and supports the finding of a NOAEL.

RELIABILITY:

1 w/o restriction	[X]
2 w restriction	[ ]
3 not reliable	[ ]
4 not assignable	[ ]

Reason: Followed recognized toxicology testing procedures and test material adequately characterized.